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National Institute of Neurological Disorders and Stroke

Spinal Cord Injury: Emerging Concepts

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Executive Summary

Among the most exciting frontiers in medicine is the repair of traumatic injuries to the **spinal cord**. Improvements in treatment are helping many more people survive **spinal cord injury**. Yet most **spinal cord** injuries still cause lifelong disability, and continued research is critically needed. To explore new directions for research on **spinal cord injury**, the National Institutes of Health sponsored a scientific workshop on September 30 - October 1, 1996. The workshop, **Spinal Cord Injury**: Emerging Concepts, brought together experts from the field of **spinal cord injury** research and leaders from other fields such as development, immunology, and stroke.

Current Understanding and Treatment

The normal **spinal cord** coordinates movement and sensation in the body. It is a complex organ containing nerve cells, supporting cells, and nerve fibers to and from the brain. The **spinal cord** is arranged in segments, with higher segments controlling movement and sensation in upper parts of the body and lower segments controlling the lower parts of the body. The consequences of **injury** reflect this organization.

The types of disability associated with **spinal cord injury** vary greatly depending on the type and severity of the **injury**, the level of the **cord** at which the **injury** occurs, and the nerve fiber pathways that are damaged. Severe **injury** to the **spinal cord** causes paralysis and complete loss of sensation to the parts of the body controlled by the **spinal cord** segments below the point of **injury**. **Spinal cord** injuries also can lead to many complications, including pressure sores and increased susceptibility to respiratory diseases.

Clinical management of **spinal cord injury** has advanced greatly in the last 50 years. Recent advances include improved imaging of damage to the **spinal cord** and vertebrae and development of the first effective drug therapy for use in the hours just after **injury**. Current management of acute **spinal cord injury** involves diagnosing and relieving gross misalignments and other structural problems of the spine, minimizing cellular-level damage, and stabilizing the vertebrae to prevent further **injury**. Once a patient is stabilized, supportive care and rehabilitation strategies promote long-term recovery.

Secondary Damage

Damage to the **spinal cord** does **not** stop immediately after the initial **injury**, but continues in the hours following trauma. These delayed **injury** processes present windows of opportunity for treatments aimed at reducing the extent of disability resulting from **spinal cord injury**.

Most types of immune cells enter the **spinal cord** only rarely. However, when the **spinal cord** is damaged by trauma or disease, immune cells engulf the area, eliminating debris and releasing a host of powerful regulatory chemicals, both beneficial and harmful. Scientists know little about the role of these immune cells after **spinal cord injury**.

Following **spinal cord injury**, highly reactive chemicals called oxidants or "free radicals" are released. These chemicals attack the body's natural defenses and critical cell structures. Trauma also causes release of excess neurotransmitters, leading to excitotoxicity, or secondary damage from overexcited nerve cells. Understanding how to block oxidative damage and excitotoxicity may provide avenues for reducing damage following **spinal cord injury**.

New insights about how cells die are affecting many areas of disease research, including **spinal cord injury**. Until recently, most cell death in **spinal cord injury** was attributed to necrosis, the common, uncontrolled form of cell death in which cells swell and break open. Recent experiments have shown that some cells die as a result of apoptosis, a form of "cell suicide" in which damaged cells eliminate themselves with less harm to their neighbors. Blocking apoptosis appears to improve recovery after **spinal cord injury** in rodents.

Damage to axons - nerve fibers that signal to other cells - causes most of the problems associated with **spinal cord injury**. Until recently, most researchers assumed that the physical forces of **spinal cord** trauma immediately tear axons. New evidence suggests that many axons deteriorate more slowly because the vital transport of molecules

and cell components to and from the ends of axons is disrupted. This delay in axon loss allows time for intervention.

Following **injury**, nerve cells in the **spinal cord** below the lesion may die, disrupting **spinal cord** circuits that help control movement and interpret sensory information. Understanding these changes will be essential for obtaining useful recovery of function following regeneration.

Regeneration

For successful regeneration to occur following **spinal cord injury**, damaged nerve cells must survive or be replaced, and axons must regrow and find appropriate targets. Axons and their targets must then interact to construct synapses, the specialized structures that act as the functional connections between nerve cells.

Although conditions in the injured adult **spinal cord** are significantly different from those occurring during development, the requirements for regeneration are similar to those for development. Scientists are beginning to learn how cells specialize, how axons find their correct targets, and how synapses form in the developing **spinal cord**. Physicians may ultimately be able to manipulate developmental signals to control regeneration.

Central nervous system neurons require combinations of natural chemicals called trophic factors to survive and grow. Understanding which trophic factors are important and how cells respond to these molecules may enable researchers to use trophic factors to foster regeneration after **spinal cord injury**. Research on ways to administer these factors and avoid side effects will be necessary before they can be used for **human spinal cord injury**. Scientists are currently studying how nerve cells' innate ability to grow, and the environment that surrounds them, affect regeneration following **injury**. For example, investigators recently discovered a gene that prevents nerve cells from growing in adults. Methods that control this innate ability to grow may eventually complement other therapies.

Researchers are beginning to apply new knowledge about regeneration in **animal** models of **spinal cord injury**. Strategies include grafting of peripheral nerve pieces and fetal tissue into the damaged **spinal cord**, administering growth factors, genetically manipulating cell death programs, and neutralizing or bypassing natural growth-inhibiting substances. Combinations of such therapies have produced the first evidence that some functional regeneration of completely severed **spinal** cords in adult mammals is possible.

Current Interventions

Effective drug therapy for **spinal cord injury** first became a reality in 1990 with the finding that the steroid drug methylprednisolone can significantly improve recovery. Clinical trials of methylprednisolone demonstrated that there is an 8-hour window of opportunity for treatment after **injury**. This trial also showed that health care systems can provide the rapid treatment necessary in **spinal cord injury**, and it serves as a model for efficient clinical trials of other therapies. Methylprednisolone has now moved from clinical trials to standard use.

Neural prostheses present another approach for improving the quality of life after **spinal cord injury**. These electronic and mechanical devices, such as hand-grasp prostheses, connect with the nervous system to supplement or replace lost motor or sensory function. Devices such as prostheses to control bladder function and to help people stand are now in development or planning stages.

Rehabilitation can greatly improve patients' health and quality of life. New knowledge about the factors underlying spasticity, muscle weakness, and incoordination may lead to innovative ways of reducing these problems. In some cases, drugs available for other purposes may be effective for treating problems associated with **spinal cord injury**.

Preclinical and Clinical Testing of New Therapies

Animal studies point to several avenues for developing new therapies for **spinal cord injury**, including drugs that promote regeneration and transplantation strategies. Each of the mechanisms of secondary damage offers targets for intervention.

Efficient preclinical tests can ensure that the most promising potential therapies proceed rapidly to clinical testing. New **animal** models, innovative approaches to testing, and reliable outcome assessments are essential to this process.

Randomized, controlled, clinical trials are the gold standard for revealing the benefits and drawbacks of a particular therapy, but practical and ethical constraints limit large-scale trials to the most promising therapies. Good preclinical data is essential so that researchers can predict which treatments and doses are most useful and which patients might benefit. Combination therapies present special challenges that must be overcome when designing clinical trials for promising therapies.

Conclusion

Spinal cord injury research has now come of age. Because of general progress in neuroscience, as well as specific advances in **spinal cord injury** research, researchers can now test new ideas about how changes in molecules, cells, and their complex interactions in the living body determine the outcome of **spinal cord injury**. One of the most exciting messages from the workshop was the confirmation that findings from other fields, such as development, immunology, and stroke research, can be applied to the study of **spinal cord injury**.

Researchers are wary of giving people false hope that a "magic bullet" for curing **spinal cord injury** is just around the corner. However, with accelerating progress in basic and applied research, there is renewed vitality and growing optimism among investigators that, with continued effort, the problems of **spinal cord injury** will be overcome.

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Introduction

As the 20th century draws to a close, advances in scientific understanding of the **human** body are leading to tremendous opportunities for treating even the most devastating diseases. Among the most exciting frontiers in medicine is the repair of traumatic injuries to the central nervous system (CNS), including the **spinal cord**. Improvements in treatment are helping many more people survive **spinal cord injury**, and the time survivors must spend in the hospital is half what it was 20 years ago. Yet most **spinal cord** injuries still cause lifelong disability, and further research is critically needed.

The **injury** of actor Christopher Reeve in 1995 drew the nation's attention to the tragedy of **spinal cord injury**. Accidents and violence cause an estimated 10,000 **spinal cord** injuries each year, and more than 200,000 Americans live day-to-day with the disabling effects of such trauma. The incidence of **spinal cord** injuries peaks among people in their early 20s, with a small increase in the elderly population due to falls and degenerative diseases of the spine. Because **spinal cord** injuries usually occur in early adulthood, those affected often require costly supportive care for many decades. The individual costs may exceed $250,000 per year, placing an often overwhelming financial burden on these individuals and their families. For the nation, these costs add up to an estimated $10 billion per year for medical and supportive care alone. Of course, no dollar figure can describe the **human** costs to **spinal cord** injured people and their families.

To explore new directions for research on **spinal cord injury**, the National Institutes of Health sponsored a scientific workshop on September 30 - October 1, 1996. The workshop, **Spinal Cord Injury**: Emerging Concepts, brought together experts from the field of **spinal cord injury** research and leaders from other fields such as development, immunology, and stroke. The organizers hoped that interactions among these experts might bring new interest and new ideas to **spinal cord injury** research and foster fruitful collaborations between investigators. Because of the remarkable progress in basic and clinical neuroscience, the time is now ripe to apply knowledge from other fields to treatment of **spinal cord injury**.

The workshop participants discussed four major topics: the current understanding and treatment of **spinal cord injury**, mechanisms of secondary damage, possibilities for regeneration, and strategies for intervention. The discussions revealed many areas where continued research could yield benefits. For example, in recent years scientists have gained a better understanding of how trauma injures nerve cells and why cells die. They know that secondary damage continues for hours following an initial trauma, presenting windows of opportunity to limit this damage. Other opportunities for therapeutic intervention, including rehabilitation strategies, extend well beyond this time window. Progress in understanding how the **spinal cord** changes after **injury** is pointing to new therapeutic approaches.

The ultimate hope, of course, is **not** just to minimize damage, but to foster recovery. A century of pessimism about the capacity for regeneration in the brain and **spinal cord** is now giving way to guarded optimism. Scientists recently demonstrated that nerve cells in the **spinal cord** can regrow under certain circumstances. Insights from **animal** models of **spinal cord injury** and from studies of nervous system development are leading to strategies that may foster regeneration. Researchers also are making outstanding progress in devising neural prostheses that can substitute for some of the functions lost after **spinal cord injury**. While it is unlikely that the complex problem of **spinal cord injury** will be solved by a single dramatic discovery, small improvements in therapy can combine to improve the quality of life for those who live with such devastating injuries.

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Current Understanding and Treatment

To understand how treatment for **spinal cord injury** can be improved, it is important to understand the normal **spinal cord** and its functions, how these functions change after **injury**, and the status of current treatment.

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The Normal Spinal Cord

The **spinal cord** and the brain together make up the CNS. The **spinal cord** coordinates the body's movement and sensation. Unlike nerve cells, or neurons, of the peripheral nervous system (PNS), which carry signals to the limbs, torso, and other parts of the body, neurons of the CNS do **not** regenerate after **injury**.

The **spinal cord** includes nerve cells, or neurons, and long nerve fibers called axons. Axons in the **spinal cord** carry signals downward from the brain (along descending pathways) and upward toward the brain (along ascending pathways). Many axons in these pathways are covered by sheaths of an insulating substance called myelin, which gives them a whitish appearance; therefore, the region in which they lie is called "white matter." The nerve cells themselves, with their tree-like branches called dendrites that receive signals from other nerve cells, make up "gray matter." This gray matter lies in a butterfly-shaped region in the center of the **spinal cord**. Like the brain, the **spinal cord** is enclosed in three membranes (meninges): the pia mater, the innermost layer; the arachnoid, a delicate middle layer; and the dura mater, which is a tougher outer layer.

The **spinal cord** is organized into segments along its length. Nerves from each segment connect to specific regions of the body. The segments in the neck, or cervical region, referred to as C1 through C8, control signals to the neck, arms, and hands. Those in the thoracic or upper back region (T1 through T12) relay signals to the torso and some parts of the arms. Those in the upper lumbar or mid-back region just below the ribs (L1 through L5) control signals to the hips and legs. Finally, the sacral segments (S1 through S5) lie just below the lumbar segments in the mid-back and control signals to the groin, toes, and some parts of the legs. The effects of **spinal cord injury** at different segments reflect this organization.

Several types of cells carry out **spinal cord** functions. Large motor neurons have long axons that control skeletal muscles in the neck, torso, and limbs. Sensory neurons called dorsal root ganglion cells, whose axons form the nerves that carry information from the body into the **spinal cord**, are found immediately outside the **spinal cord**. **Spinal** interneurons, which lie completely within the **spinal cord**, help integrate sensory information and generate coordinated signals that control muscles. Glia, or supporting cells, far outnumber neurons in the brain and **spinal cord** and perform many essential functions. One type of glial cell, the oligodendrocyte, creates the myelin sheaths that insulate axons and improve the speed and reliability of nerve signal transmission. Other glia enclose the **spinal cord** like the rim and spokes of a wheel, providing compartments for the ascending and descending nerve fiber tracts. Astrocytes, large star-shaped glial cells, regulate the composition of the fluids that surround nerve cells. Some of these cells also form scar tissue after **injury**. Smaller cells called microglia also become activated in response to **injury** and help clean up waste products. All of these glial cells produce substances that support neuron survival and influence axon growth. However, these cells may also impede recovery following **injury**.

Nerve cells of the brain and **spinal cord** respond to insults differently from most other cells of the body, including those in the PNS. The brain and **spinal cord** (i.e., the CNS) are confined within bony cavities that protect them, but also render them vulnerable to compression damage caused by swelling or forceful **injury**. Cells of the CNS have a very high rate of metabolism and rely upon blood glucose for energy. The "safety factor," that is the extent to which normal blood flow exceeds the minimum required for healthy functioning, is much smaller in the CNS than in other tissues. For these reasons, CNS cells are particularly vulnerable to reductions in blood flow (ischemia). Other unique features of the CNS are the "blood-brain-barrier" and the "blood-**spinal-cord** barrier." These barriers, formed by cells lining blood vessels in the CNS, protect nerve cells by restricting entry of potentially harmful substances and cells of the immune system. Trauma may compromise these barriers, perhaps contributing to further damage in the brain and **spinal cord**. The blood-**spinal-cord** barrier also prevents entry of some potentially therapeutic drugs. Finally, in the brain and **spinal cord**, the glia and the extracellular matrix (the material that surrounds cells) differ from those in peripheral nerves. Each of these differences between the PNS and CNS contributes to their different responses to **injury**.

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Anatomical and Functional Changes After Injury

The types of disability associated with **spinal cord injury** vary greatly depending on the severity of the **injury**, the segment of the **spinal cord** at which the **injury** occurs, and which nerve fibers are damaged. In **spinal cord injury**, the destruction of nerve fibers that carry motor signals from the brain to the torso and limbs leads to muscle paralysis. Destruction of sensory nerve fibers can lead to loss of sensations such as touch, pressure, and temperature; it sometimes also causes pain. Other serious consequences can include exaggerated reflexes; loss of bladder and bowel control; sexual dysfunction; lost or decreased breathing capacity; impaired cough reflexes; and spasticity (abnormally strong muscle contractions). Most people with **spinal cord injury** regain some functions between a week and six months after **injury**, but the likelihood of spontaneous recovery diminishes after six months. Rehabilitation strategies can minimize the long-term disability.

Spinal cord injuries can lead to many secondary complications, including pressure sores, increased susceptibility to respiratory diseases, and autonomic dysreflexia. Autonomic dysreflexia is a potentially life-threatening increase in blood pressure, sweating, and other autonomic reflexes in reaction to bowel impaction or some other stimulus. Careful medical management and skilled supportive care is necessary to prevent these complications.

Researchers studying **spinal** cords obtained from autopsy have identified several different types of **spinal cord** injuries. The most common types of **spinal cord** injuries found in one large study were contusions (bruising of the **spinal cord**) and compression injuries (caused by pressure on the **spinal cord**). Other types of **injury** included lacerations, caused by a bullet or other object, and central **cord** syndrome.

In **contusion** injuries, a cavity, or hole, often forms in the center of the **spinal cord**. Myelinated axons typically survive in a ring along the inside edge of the **cord**. Some axons may survive in the center cavity, but they usually lose their myelin covering. This demyelination greatly slows the speed of nerve transmission. Slowing of nerve impulses can be measured by a diagnostic technique called transcranial magnetic stimulation (TMS).

Another example of a **spinal cord injury** is central **cord** syndrome, which affects the cervical (neck) region of the **cord** and results from focused damage to a group of nerve fibers called the corticospinal tract. The corticospinal tract controls movement by carrying signals between the brain and the **spinal cord**. Patients with central **cord** syndrome typically have relatively mild impairment, and they often spontaneously recover many of their abilities. Patients usually recover substantially by 6 weeks after **injury**, despite continued loss of axons and myelin. Delays in motor responses persist, but permanent impairment is usually confined to the hands.

Complete severing of the **spinal cord** is rare in humans, but even axons that survive the initial **injury** often lose their ability to function. Secondary damage, which continues for hours, can cause loss of myelin, degeneration of axons, and nerve cell death. Patients with their **spinal** cords completely severed often show abnormal reflexes that emerge more than 8 months after **injury**. These reflexes, such as twitching of muscles in the arm and hand in response to sensory stimulation of the legs and feet, may result from "sprouting" of new branches from sensory fibers just below the lesion. They may also result from activation of nerve pathways that are normally suppressed. Other abnormal responses, such as sweating in response to movement of a hair, may be due to sprouting of nerves in the autonomic nervous system. The autonomic nervous system is the part of the PNS that controls involuntary body functions such as sweating and heart rate.

Since even a small number of nerve fibers can support significant nervous system function, measures that reduce damage could allow much greater function than would otherwise be expected. Devising interventions that will achieve this goal is one of the major challenges in **spinal cord injury** research today.

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Clinical Management

Medical care of **spinal cord injury** has advanced greatly in the last 50 years. During World War II, **injury** to the **spinal cord** was usually fatal. While postwar advances in emergency care and rehabilitation allowed many patients to survive, methods for reducing the extent of **injury** were virtually unknown. Although techniques to reduce secondary damage, such as **cord** irrigation and cooling, were first tried 20 to 30 years ago, the principles underlying effective use of these strategies were **not** well understood. Significant advances in recent years, including an effective drug therapy for acute **spinal cord injury** (methylprednisolone) and better imaging techniques for diagnosing **spinal** damage, have improved the recovery of patients with **spinal cord** injuries.

Current care of acute **spinal cord injury** involves three primary considerations. First, physicians must diagnose and relieve **cord** compression, gross misalignments of the spine, and other structural problems. Second, they must minimize cellular-level damage if at all possible. Finally, they must stabilize the vertebrae to prevent further **injury**.

The care and treatment of persons with a suspected **spinal cord injury** begins with emergency medical services personnel, who must evaluate and immobilize the patient. Any movement of the person, or even resuscitation efforts, could cause further **injury**. Even with much-improved emergency medical care, many people with **spinal cord injury** still die before reaching the hospital.

Methylprednisolone, a steroid, has become standard treatment for acute **spinal cord injury** since 1990, when a large-scale clinical trial showed significantly better recovery in patients who began treatment with this drug within 8 hours of their **injury**. Methylprednisolone reduces the damage to cellular membranes that contributes to neuronal death after **injury**. It also reduces inflammation near the **injury** and suppresses the activation of immune cells that appear to contribute to neuronal damage. Preventing this damage helps spare some nerve fibers that would otherwise be lost, improving the patient's recovery.

A controversial topic in the acute care of **spinal cord injury** is whether surgery to reduce pressure on the **spinal cord** and stabilize it is better than traction alone. A study in the 1970s showed that, in some cases, surgical intervention actually worsened the patient's condition. This finding prompted many physicians to become more conservative about using these techniques, although advances in care since that time have reduced the risk of complications due to surgery. While there is no proof that surgeons must operate to decompress the **spinal cord** within the 8-hour time window established for methylprednisolone, many believe it may help and try to do it then. Early surgery also allows earlier movement and earlier physical therapy, which are important for preventing complications and regaining as much function as possible. Use of imaging methods such as computed tomography (CT) scans to visualize fractures and magnetic resonance imaging (MRI) to image contusions, disc herniation, and other damage can help define the appropriate treatment for a particular patient. Several types of metal plates, screws, and other devices also are now available for surgically stabilizing the spine.

Once a patient's condition is stabilized, care and treatment focus on supportive care and rehabilitation strategies. Attention to supportive care can prevent many complications. For example, periodically changing the patient's position can prevent pressure sores and respiratory complications. Rehabilitation, which focuses on the patient's physical and emotional recovery, is also very important. Almost all patients with **spinal cord** injuries can now achieve a partial return of function with proper physical therapy that maintains flexibility and function of the muscles and joints. Physical therapy can also help reduce the risk of blood clots and boost the patient's morale, while counseling can help a person adjust emotionally to the **injury** and its consequences.

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Conclusion

Recent years have seen many advances in understanding and treating **spinal cord injury**. These include the development of CT and MRI scans to visualize injuries and the use of methylprednisolone to reduce damage. However, many facets of what happens when the **spinal cord** is injured are still unknown. An exact description of the structural and tissue changes that occur in **spinal cord injury** is necessary for planning effective interventions. Studies aimed at better describing what happens following **spinal cord injury** may lead to improved treatments.

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Secondary Damage

Damage to the **spinal cord** does **not** stop with the initial **injury**, but continues in the hours following trauma. Paradoxically, this delayed, secondary damage is **not** all bad news because secondary **injury** processes present windows of time in which treatment may reduce the extent of disability. The effects of methylprednisolone demonstrate that such treatment is possible and present a model for the development of other treatments.

Two major themes about secondary damage recurred throughout the workshop. The first theme reflects increasing recognition that similar cellular processes contribute to damage in many different neurological disorders. The second theme mirrors one of the most active areas in all of biology -- how cells die. Cells, including those in the **spinal cord**, die in two general ways. Necrosis is a relatively uncontrolled process in which cells swell and break open, leaking substances that can be toxic to their neighbors. However, in apoptosis, or programmed cell death, cells activate a "cell suicide" program, an ordered sequence of events that leads to cell death with relatively little damage to surrounding cells. The relationship between apoptosis and necrosis, the role that each plays in **spinal cord injury**, the signals that regulate cell death, and the potential to halt death programs are now being explored to find ways of minimizing secondary damage following **spinal cord injury**.

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Immune System Reactions

There is no single point at which to begin describing the intricately intertwined cellular and molecular events that follow **spinal cord injury**. However, the immune reaction is a good place to start because of its importance. Most types of immune cells enter the CNS only rarely unless it has been damaged by trauma or disease. It is **not** always clear to what extent immune reactions help or harm prospects for recovery, although immune reactions do appear to cause some secondary damage.

The last decade has brought extraordinary advances in understanding the immune system and its interactions with the nervous system. Using newly developed markers, scientists can identify subsets of immune cells with different functions and can monitor these cells in the nervous system. They are also beginning to understand the chemical language immune cells use to communicate. Cytokines, for example, are a diverse group of diffusible messenger molecules that control many aspects of immune cell function and also enable immune cells to influence other cells such as neurons. Cell adhesion molecules on the surfaces of cells control the traffic of immune cells into the brain and **spinal cord** and have other wide-ranging influences. Epithelial cells of blood vessels and various types of immune cells normally display certain cell adhesion molecules on their surface. These adhesion molecules change when blood vessel and immune cells encounter foreign molecules, sense damaged tissue in the vicinity, or detect cytokines. Advances in understanding the immune system are now being applied to learn how immune cells influence recovery from **spinal cord injury**.

Microglial cells, which are normally found in the CNS, have some immune functions and become activated in response to damage. Following trauma, other types immune cells react to signals from damaged tissue and changes in endothelial cells by entering the CNS. Neutrophils are the first type of immune cells to enter the CNS from the rest of the body. These cells enter the **spinal cord** within about 12 hours of **injury** and are present for about a day. About 3 days after the **injury**, T-cells enter the CNS. T cells have many functions in the body, including killing infected cells and regulating many aspects of the immune response; however, their function in **spinal cord injury** is totally unknown. The key types of immune cells in **spinal cord injury** appear to be macrophages and monocytes, which enter the CNS after the T-cells. These cells scavenge cellular debris. One type of macrophage, the perivascular cell, may also mediate damage to the endothelial cells that line blood vessels. It is **not** clear which signals control the entry of immune cells into the CNS, but changes in cell adhesion molecules most likely play an important role.

What immune cells do once they enter the damaged **spinal cord** is poorly understood. Some cells engulf and eliminate debris as they do during inflammation in other parts of the body. Macrophages, monocytes, and microglial cells release a host of powerful regulatory substances that may help or hinder recovery from **injury**. Potentially beneficial substances released by these cells include the cytokines TGF-beta and GM-CSF (transforming growth factor-beta and granulocyte-macrophage colony-stimulating factor) and several other growth factors. Apparently detrimental products include cytokines such as TNF-alpha and IL-1-beta (tumor necrosis factor-alpha and interleukin-1-beta) and chemicals such as superoxides and nitric oxide that may contribute to oxidative damage. Again, it is unclear what is helpful and harmful about many of these powerful substances in the context of the injured **spinal cord**.

Several workshop participants emphasized how important it is to learn about the role of the immune response in **spinal cord injury**. Understanding the possible links between the immune system and oxidative damage, apoptosis of nerve cells, and demyelination is an important area for research. Other critical areas for study include the signals controlling the traffic of immune cells into the **spinal cord** following **injury** and the time course and subsets of immune cells involved. Progress in understanding the immune system now makes answering these questions technically possible.

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Oxidative Damage

After a **spinal cord injury**, the body's inflammatory cells, among others, produce highly reactive oxidizing agents including "free radicals." Oxidizing agents attack molecules that are crucial for cell function by modifying their chemical structures. This process is called oxidative damage. Oxidative damage occurs in disorders ranging from slow neurodegenerative diseases like amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) and Parkinson's disease to acute events like stroke and trauma. Thus, it has been the focus of intensive research. Scientists are learning which chemicals are responsible for oxidative damage in the nervous system, how they are generated, and what role the natural antioxidant defense systems play.

Free radicals are produced as a byproduct of normal metabolism. The brain and **spinal cord** normally have a high rate of oxidative (energy-producing) metabolism. The increases in blood flow during "reperfusion," when blood flow is restored following **injury**, may raise free radical production even more. Inflammation can also accelerate the production of free radicals. Many scientists believe that superoxides (oxygen molecules with an extra electron) can escape from the normal antioxidant defenses of the CNS and combine with hydrogen peroxide, also normally present, to form hydroxyl radicals (oxygen-hydrogen with an extra electron). In the test tube, hydroxyl radicals are extremely reactive and quickly attack crucial cellular structures and enzymes. However, evidence suggests that this scenario may be different in the living CNS. For one thing, the CNS has concentrations of enzymes that can safely inactivate free radicals. The antioxidant enzyme called copper-zinc superoxide dismutase (SOD), for example, is abundant in the CNS.

Although hydroxyl radicals are the most reactive molecules in the test tube, nitric oxide may be a more important cause of oxidative damage in living animals. Nitric oxide itself is **not** very destructive -- in fact the body uses it as a signaling molecule -- but it can combine with superoxide ions to produce a very toxic compound called peroxynitrite. Nitric oxide forms peroxynitrite by a reaction that is a million times faster than the one that forms hydroxyl radicals, and it diffuses ten thousand times farther. Peroxynitrite increases its range of damage even more by inactivating some antioxidant defenses, such as SOD. This free radical also can change how cells respond to natural growth and survival factors; for example, it can change the effect of NGF (nerve growth factor) from protecting against apoptosis to accelerating this type of cell death.

The complex actions of nitric oxide illustrate how the interactions between oxidants and biological systems influence how toxic the oxidants' effects can be. These results focus attention on harmful chemical agents that elude antioxidant defenses and attack critical cell molecules. One useful finding is that nitric oxide damage leaves a characteristic molecular "footprint" on cell proteins. This footprint may allow researchers to identify targets of oxidative damage following **spinal cord** trauma and help in developing therapeutic and protective measures.

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Calcium and Excitotoxicity

Following trauma, an excessive release of neurotransmitters - chemical messengers that travel between neurons -- can cause secondary damage by overexciting nerve cells. This phenomenon, called excitotoxicity, has been a major focus of research on stroke and traumatic brain **injury**, and it may also contribute to neurodegenerative diseases and **spinal cord injury**. Researchers know about excitotoxicity (and calcium-mediated damage, which often follows) from both cell culture experiments, in which relevant variables are simplified and controlled, and from experiments in the much more complex living animals. Insights about excitotoxicity are now being applied to understanding secondary damage following **spinal cord** trauma.

Glutamate is the neurotransmitter most often used by nerve cells to activate, or excite, one another. Excitotoxicity caused by excessive release of glutamate contributes to damage following traumatic CNS **injury** and stroke. Excessive glutamate can damage nerve cells and glia in several ways. One harmful sequence begins when glutamate overactivates a type of glutamate receptors called NMDA receptors, allowing high levels of calcium to enter the cell. Calcium regulates many cellular processes. For example, calcium activates certain proteases called calpains. Proteases are enzymes that degrade other proteins and have important regulatory roles in cells. Inappropriate activation of these enzymes can damage important parts of the cell. Calcium metabolism is intimately related to oxidative damage as well. Mitochondria--structures within cells that are responsible for producing energy by oxidation -- actively take up calcium. Mitochondria damaged by excessive calcium may produce even more oxidizing free radicals. Excitotoxicity can also damage cells through processes that do **not** involve calcium. For example, glutamate allows entry into cells of ions such as sodium and chloride that can cause water to enter, leading to uncontrolled swelling.

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Necrosis and Apoptosis

New insights about how cells die are dramatically affecting many areas of disease research, and **spinal cord injury** is no exception. Until recently, scientists believed that necrosis, or uncontrolled cell death, was the only way cells die after CNS trauma. Findings presented at the workshop now suggest that apoptosis (programmed cell death) occurs in parallel with necrosis, and that delayed apoptosis contributes to secondary damage following **spinal cord** trauma.

Cell death programs and **experimental** interventions to halt them were major themes of the workshop.

Apoptosis occurs in many contexts other than disease. For example, it plays a key role in the developing nervous system. The embryonic **spinal cord** and brain generate many more neurons than are found in the adult organism. Neurons compete for natural chemicals called trophic factors that are supplied by target cells, and nerve cells that do **not** make proper connections die by apoptosis.

Many forms of damage can trigger cell death. Cells undergoing apoptosis exhibit changes very different from those of cells dying from necrosis, reflecting the more controlled nature of programmed cell death. Necrotic cells swell and break open, leaking their contents into the surrounding area and provoking an inflammatory response. In apoptosis, cells go through a series of characteristic structural changes. During apoptosis, bubbles or "blebs" form in the outer cell membrane, and membrane-enclosed fragments of the cell may break away. The cell nucleus also condenses and fragments, and polyribosomes (the cellular machinery for synthesizing proteins) break up. In most cells, enzymes cut DNA into unequal pieces. This DNA degradation may have evolved as a defense against viruses that attempt to establish residence within cells. Chemicals released from dying cells then induce surrounding cells to scavenge the debris. Apoptosis eliminates damaged cells without releasing dangerous molecules like proteases and glutamate that might harm neighboring cells.

It is **not** obvious that preventing apoptosis would be beneficial in **spinal cord injury**. Cells rescued from apoptosis might go on to die by necrosis and damage their neighbors. Nerve cells that survive a "suicide attempt" might have impaired function and be more disruptive than beneficial. In many cases, necrosis and apoptosis probably occur in parallel. In experiments reported at the workshop, necrosis from excitotoxicity killed most cultured cells from the mouse cerebral cortex. Blocking this excitotoxic necrosis with glutamate antagonist drugs and extending oxygen-glucose deprivation to overcome the protective effect led to apoptosis. Some drugs had opposite effects on necrosis and apoptosis. For example, certain chemical signals promoted necrosis but reduced apoptosis.

Recently, scientists have found that apoptosis contributes to **spinal cord** cell death and dysfunction after trauma. Necrosis was prominent in rats subjected to severe **spinal cord** trauma. However, following milder trauma, cells died by apoptosis. Mapping the positions of apoptotic cells within these **spinal** cords revealed interesting patterns. Apoptosis of nerve cells was largely restricted to sites near the impact zone itself and generally occurred within about 8 hours of the trauma. Apoptosis in glial cells was much more prolonged, and a second wave of apoptosis occurred in the white matter -- probably among oligodendrocytes -- at about 7 days after **injury**. This wave of secondary death rippled out much further than the original site of **injury**. In one experiment, moderate-impact contusions in the rat **spinal cord** caused little apparent structural damage to myelinated axons in the first few hours, but led to extensive demyelination, probably because of delayed apoptosis of oligodendrocytes, by 3 weeks after **injury**. These results are important in defining the time windows during which therapeutic intervention might be beneficial. Optimal strategies for saving nerve cells may be different from optimal strategies for saving oligodendrocytes.

Much of what we know about the cellular mechanisms that underlie apoptosis comes from studies of the nematode worm C. elegans. This tiny worm has only about 300 nerve cells, each of which is individually recognizable, unlike the uncountable billions of neurons in a mammalian nervous system. These worms also allow genetic manipulations that are much more difficult to perform in mammals. Scientists studying C. elegans have begun to understand the basic elements of the cell death program by observing worms with mutations in genes that control apoptosis. These include death-suppressor genes, killer genes, genes that control engulfment of cell debris, and genes for degrading DNA. Crucial cellular processes are highly conserved in evolution, that is, they don't change much between lower and higher animals. The detection of cell death genes in higher organisms, based on their resemblance to genes in worms, has been key to understanding cell death in mammals.

The best-studied models of mammalian nerve cell apoptosis are cultures of sympathetic nerve cells (a type of PNS cell) from which the critical trophic factor NGF, or nerve growth factor, has been removed. The cell death program initiated by removing NGF includes five stages: activation, propagation, commitment, execution, and death. Scientists have now defined each stage by cellular events such as the activation of specific genes and enzyme systems. Up until the commitment stage, interrupting the synthesis of new proteins needed for the program to proceed can halt apoptosis. Even after that stage, blocking the actions of certain enzymes, especially a group of protein-degrading enzymes called the ICE (interleukin converting enzyme) family of proteases, can interrupt the death program. Cell death programs may differ among cells; for example, some cells apparently do **not** require new protein synthesis for apoptosis. Different cell death programs may occur even in the same type of nerve cell in response to different types of **injury**. In all cases, however, the cells actively participate in the process that leads to their demise.

Using cultured PNS neurons, scientists have tested two strategies for interrupting programmed cell death. One method used drugs that inhibit the ICE family of proteases, proteins that are crucial for the cell death program. The other method used genetically engineered cells lacking bcl-2, a regulator gene needed for the apoptosis program to

go forward. In other words, scientists bred mice in which the cell death program was genetically suppressed. Scientists found that regardless of the strategy tested, nerve cells deprived of NGF were arrested in a metabolically quiescent "undead state" for long periods. When subsequently given NGF, these cells were "resurrected" -- they appeared normal and grew.

Similar strategies have been used to block apoptosis in **animal** models of cerebral ischemia (stroke) and **spinal cord injury**. In rodent models of stroke, blocking apoptosis, either with drugs or by genetic manipulations, reduced brain damage after blood flow was interrupted. Improved movement in these animals showed that surviving brain cells could still function. Rats with **spinal cord** injuries that were given an inhibitor of protein synthesis for 1 month were able to retain some use of their hindlimbs. This radical treatment blocked apoptosis by preventing the synthesis of new proteins necessary for the cell death program to go forward.

These studies collectively suggest that blocking cell death programs might buy time that will allow some cells to survive the initial trauma of **spinal cord injury**. However, the methods used to block cell death in these experiments are **not** practical for **human** application: The drugs can be toxic, and genetic manipulation to create humans resistant to **injury** is obviously **not** a viable solution. In addition to developing better drugs to block apoptosis, scientists need to answer several key questions about the nature of cell death. These questions include what triggers apoptosis, how developmental apoptosis resembles (or differs from) **injury**-related apoptosis, how cell death programs and timing vary in different cell types, and to what extent this form of cell death contributes to the functional losses seen in patients with **spinal cord injury**.

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Axon Damage

With the current scientific excitement about cell death, it is important to emphasize that damage to axons causes most of the problems associated with **spinal cord injury**, including loss of motor control and sensation. In rat **spinal cord contusion** injuries, for example, recovery of function correlates closely to the number of remaining axons. Until recently, most researchers assumed that the physical forces of **spinal cord** trauma immediately tear axons. Recent studies of axon damage following traumatic brain **injury** are changing this view.

Within several days of traumatic brain or **spinal cord injury**, grossly swollen axons, termed "reactive swellings" or "retraction balls," appear. Many scientists believe that physical forces of trauma stretch axon fibers, causing them to tear and swell. Studies using multiple **animal** models and various anatomical tracers now have shown that much of the axon damage following CNS trauma is **not** immediate. Instead, it occurs hours later from swelling caused by impaired axonal transport. Axonal transport is a vital cellular process that moves molecules and cell components from the cell body toward the axon terminal and from the terminal back to the cell body.

What disrupts axonal transport and causes delayed axon damage? There appear to be multiple causes, but changes to the cytoskeleton play a critical role. The cytoskeleton is the internal scaffolding that determines the shapes of cells. It is necessary for transport of substances along the axons. In severe injuries, changes in the cell membrane that surrounds axons can allow an abnormal influx of ions, particularly calcium. This leads to compacting of the cytoskeleton and interruption of axonal transport. Calpain, a calcium-activated protein-degrading enzyme, probably participates in this process. Swelling and disrupted transport also occur in axons whose membranes show no change in ion permeability. In these axons, which predominate in mild to moderate injuries, neurofilaments (one component of the cytoskeleton) become misaligned. This, again, impairs transport and leads to swelling of axons.

Damage to axons has several consequences within the **spinal cord**. Following axon **injury**, axons disconnected from their nerve cell bodies disintegrate by a process called "Wallerian" or "orthograde" degeneration. Nerve cell bodies with damaged axons, and the axon segment that remains attached, may die by retrograde degeneration, that is, degeneration that begins at the site of **injury** and progresses back toward the cell body. From a functional point of view, the delayed death of oligodendrocytes and the resulting demyelination of axons are also critical events, because unmyelinated axons do **not** conduct electrical impulses normally. The death of these glial cells may result partly from the degeneration of damaged axons because oligodendrocytes apparently require contact with axons to remain healthy. The removal of normal nerve connections also has important consequences. The diverse effects of axon **injury** suggest that more than one therapeutic approach may be needed to overcome this damage.

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Changes Below the Injury

While the most dramatic changes in the **spinal cord** occur at or near the site of **injury**, the **spinal cord** also changes below that point. Understanding these changes is becoming more important as researchers learn how to foster axon regeneration. A key question is what regenerating axons will find when they reach the **spinal cord** below the **injury**. Changes below the **injury** site also influence clinical symptoms, such as reflex changes and spasticity, and they may be a factor in the success of future neural prostheses that might rely upon **spinal** reflexes or motor control circuits.

The **spinal cord** is **not** just a passive conduit carrying signals to and from the brain. It helps to control movement and to interpret sensory information flowing in from the body. Walking, for example, includes three neural processes. First, networks of nerve cells within the **spinal cord** (central pattern generators) generate the basic motor patterns that activate muscles in the sequence appropriate for walking. Second, sensory feedback from the limbs into the **spinal cord** modifies this basic motor pattern. Third, control signals from higher centers in the brain modulate the **spinal** circuits. These higher centers turn the **spinal** pattern generators on and off, shift between different types of locomotion, control sensory influences according to the type of movements, and govern posture and balance. Scientists are beginning to learn how these systems work and how they come together during development.

How the **spinal cord** circuitry below the trauma site changes following **injury** is poorly understood, but scientists are beginning to recognize that these changes are important. In one series of experiments, scientists transected the **spinal** cords of chick embryos and removed a segment. **Spinal** cords in very young chick embryos regenerated remarkably well. In older embryos, however, axons did **not** regenerate and many motor neurons, interneurons, and sensory neurons died below the **injury**. This cell death resulted from the **injury** rather than from the programmed cell death that normally occurs during development. These experiments suggest that death of cells below the site of **injury** may be a factor in **human spinal cord injury** as well.

Spinal cord injury also may alter connections among nerve cells that survive below the **injury**. The adult CNS is much more plastic, or changeable, than scientists believed just a few years ago. One interesting discovery is that some of the cellular mechanisms that allow the nervous system to adapt with experience, such as glutamate signaling and calcium-mediated events, are the same as those that go awry after **injury** and cause secondary damage. This discovery may explain why some of these apparently harmful mechanisms have persisted in evolution.

Immediately after **spinal cord** trauma, nerve cells below the site of **injury** are excited by trauma-induced release of neurotransmitters. A loss of normal excitatory and inhibitory signals follows when the severed axons die. In many parts of the CNS, including the **spinal cord**, strong excitation of neurons modifies the strength of synapses. This form of plasticity might alter the remaining circuits of the **spinal cord** in unpredictable ways. The removal of normal signals also provokes sprouting of nearby axons into the territory vacated by degenerating axons. The consequences of this rewiring are hard to predict. They may include the changes in reflexes often seen in people with **spinal cord injury**. These complex and diverse consequences suggest that attention to the changes below the site of **spinal cord injury** may be essential for successful regeneration and rehabilitation.

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Conclusion

Scientists who have been studying **spinal cord injury** for many years say that **spinal cord injury** research has now come of age. Because of progress in neuroscience, as well as in **spinal cord injury** research, researchers can test specific ideas about how changes in cells and molecules affect **spinal cord injury**. **Not** long ago, only descriptive studies were possible. Oxidative free radicals, calcium-mediated damage, proteases, cytoskeletal dysfunction, excitotoxicity, immune reactions, apoptosis, and necrosis all come into play following **spinal cord injury**. These sources of secondary damage interact in complex ways that scientists are just beginning to understand. What is encouraging is that each of these harmful processes offers targets for developing therapies.

Much of the workshop discussion about secondary **injury** processes relied upon experiments in fields other than **spinal cord injury**, especially stroke and traumatic brain **injury**. The potential for application of such findings to **spinal cord injury** was one of the most exciting aspects of the workshop. While scientists do **not** agree about how directly this information will apply to the specifics of **spinal cord** trauma, most believe that studying other disorders can provide insights that will improve understanding of **spinal cord injury**. Most importantly, studies in other **experimental** systems can provide hypotheses to test in models of **spinal cord injury** itself.

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Regeneration

For successful regeneration to occur following **spinal cord injury**, several things must happen. First, damaged nerve cells and supporting cells must survive or be replaced, despite the acute effects of trauma and the conspiracy of processes that cause secondary damage. Replacement of lost cells in the CNS is unlikely without intervention because adult nerve cells in the brain and **spinal cord** cannot divide. Nerve cells that survive the **injury** often must regrow axons, despite tissue changes such as cavity formation that obstruct growth. Axons also must navigate among the myriad possibilities to find appropriate targets. Once the axons locate their targets, they must construct specialized structures to release neurotransmitters at synapses, while target cells must assemble and precisely locate the structures needed to respond to neurotransmitters. Finally, the neural circuitry may have to compensate for changes that have occurred in the **spinal cord** circuitry following **injury**.

Until recently, most scientists believed that nerve cells in the CNS of adult mammals could **not** regenerate. Dramatic findings, some presented at this workshop, are now changing that pessimistic outlook. For example, some studies have demonstrated that nerve cells in the brain and **spinal cord** make unsuccessful attempts to regenerate and can regrow under some conditions. New findings also demonstrate that the **spinal cord** has more active repair mechanisms than previously suspected. Although researchers recognize the many obstacles to obtaining regeneration in the **human spinal cord**, they believe successful regeneration of even a small percentage of nerve fibers will produce significant recovery of function.

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Implications of Development

Scientists favor the **spinal cord** for studying the CNS because it is simpler than the brain. The long tradition of anatomical and physiological research on the **cord** provides a solid framework for studying development. Developing nerve cells perform the same steps needed for regeneration -- they grow, navigate, and make appropriate connections. Regenerating nerve fibers face problems that are quite different from those faced by developing nerve cells, however. For example, the tissue through which axons move is more loosely connected during development, and an injured **spinal cord** may become quite disorganized near the **injury** site. Also, distances in the adult CNS are much greater than in the embryo, and chemical signposts for navigating axons may have changed in the adult. While the extent to which regeneration resembles development is uncertain, research about nervous system development is a source of crucial insights about how to promote regeneration following **spinal cord injury**.

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Nerve Cell Differentiation

The adult **spinal cord** is an intricate assembly of cells and nerve fibers arrayed in specific locations with very precise interconnections. Nerve cells in the **spinal cord** include several types of motor neurons, sensory neurons, and interneurons, each of which varies in shape, electrical activity, neurotransmitter release, and many aspects. Glial cells also include several specialized types of cells in the mature CNS, and the major nerve pathways of the **spinal cord** white matter are highly organized anatomically. How all of this comes about has been a subject of speculation and experiments for more than a century. The mystery is finally giving way to traditional neuroscience research methods, augmented by new technologies such as molecular genetics.

The factors causing cell types in the **spinal cord** to become distinct from one another are cell lineage (which cells arise from which by cell division) and cues from within the developing embryo. Research is now identifying these chemical cues and discovering how cells respond to them. Two major signaling systems control the fate of embryonic brain and **spinal cord** cells. One system controls the specialization of the nervous system along the long axis from the brain down through the **spinal cord**. The other system controls specialization along the dorsoventral plane, that is, in a cross-section of the **spinal cord** ("dorsal" refers to the back portion and "ventral" denotes the abdominal direction). So far, the general operating principles of the two systems appear to be the same.

The control of cell identity along the dorsoventral axis of the **spinal cord** illustrates how these developmental systems operate. Among the essential tools scientists developed to study this process are chemical markers that stain specific cell types before they fully specialize in the embryonic **spinal cord**. Three cell types form in the ventral part of the early embryonic **spinal cord**. Glial cells form in the most ventral part, called the floor plate; motor neurons and interneurons form more dorsally. Experiments have shown that the key signal that determines the fate of all three cell types is a protein called sonic hedgehog. (The name arises because this mammalian molecule was

identified by its resemblance to the "hedgehog" protein of the fruitfly. Flies with a mutation in the hedgehog gene have a peculiar prickly appearance.) To simulate the situation in the developing embryo, scientists placed pieces of ventral **spinal cord** in cell culture and exposed them to different concentrations of sonic hedgehog protein. These pieces produced motor neurons, glia, or interneurons depending on the concentration of protein to which they were exposed. In the embryo, a structure called the notochord releases the sonic hedgehog protein signal. **Spinal cord** cells that lie closest to the notochord are exposed to the highest concentration of the signal and become glial cells. Those in more dorsal positions are exposed to lesser concentrations and become, respectively, motor neurons and interneurons.

Although scientists are rapidly identifying the signals that drive the generation of cell types in the developing **spinal cord**, many basic questions remain. Many signals have yet to be discovered, and it is **not** yet clear how cells sense small differences in concentrations and respond to become specialized cell types. Interactions among the various signaling systems are likewise obscure.

Answers to these questions may have implications for **spinal cord** regeneration. In the last few years, scientists have discovered that even the mature CNS may harbor latent progenitor cells that can divide and specialize to form new nerve and glial cells. In a rat model of **spinal cord** trauma, the single layer of cells lining the central canal of the **spinal cord** expands to multiple layers of cells about 48 hours after a **contusion** lesion. The central canal is continuous with the brain ventricles, large fluid-filled spaces inside the brain. During development, new nerve cells arise from cells lining these structures. Cells from the expanded central canal of injured animals appear to stream out into the **spinal cord**; these may be neural progenitor cells attempting to repair damaged tissue. It is important to know whether developmental signals that might guide neuron growth persist in the adult. Another reason studies of cell specialization may be relevant to **spinal cord injury** is that the molecules involved in this developmental process may have other important functions in the adult. Understanding the signals that control cell specialization in development may be critical for learning how to help them repair damaged **spinal** cords.

Many new findings presented at the workshop reflected the ways researchers now study the molecular machinery by which cells operate. Knowing the genetic code for proteins allows scientists to detect similarities among proteins. By comparing genes among different species, researchers can rapidly apply insights from lower organisms to mammals. Comparing newly identified genes and proteins to known ones within the same **animal** can also help scientists understand what a newly discovered protein does. Identifying one protein often helps reveal other members of the same protein family that have related functions, as in families of growth factors, cell adhesion molecules, and neurotransmitter receptors. Scientists are also learning to recognize functional regions that many proteins share in different combinations. Gene sequences predict many aspects of a protein's function, such as whether it will respond to certain regulator molecules. Thus, the chemical language that orchestrates development provides crucial clues about regeneration, even if the processes differ.

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Axon Pathfinding

Developing nerve cells of the brain and **spinal cord** grow axons over long distances, along specific routes, and to precise targets. The tip of a growing axon forms a specialized structure called a growth cone. These growth cones sense cues, integrate that information, and make choices that steer the axon in one direction or another. Scientists have identified attractants and repellents that diffuse over long distances, as well as chemical attractants and repellents with fixed locations. Together, these cues allow axons to navigate through the developing brain and **spinal cord**. The identification of one family of long-distance attractants, the netrins, illustrates this area of research and its potential relevance to **spinal cord** regeneration.

A century ago, the Spanish neuroanatomist Ramón y Cajal speculated that diffusible chemical signals might guide growing axons. The first such signals, called netrins, were discovered just a few years ago in the chick **spinal cord**. "Commissural" neurons in the dorsal part of the **spinal cord** send axons from the front of the **cord** around toward the back. When the growth cones of these axons approach the midline of the developing **spinal cord**, they make a beeline for the floor plate, a specialized region of the embryonic **spinal cord** at the ventral edge of the midline. When scientists placed pieces of developing **spinal** cords in various arrangements in culture, they found that something in the ventral floorplate attracted growing commissural axons from a distance. They isolated the attractants and named the identified proteins netrins. When scientists further examined the effects of netrins, they found that these molecules also repelled growing axons from other types of developing nerve cells. This finding was predicted by studies in worms of molecules that closely resemble netrins. Experiments in normal and mutant mice confirmed that these molecules guide developing axons in living mammals.

Many guidance molecules were only recently identified, and certainly more remain to be found. Similarities between

guidance molecules in mammals and those in simple organisms like worms and fruitflies are speeding progress in this area of neurobiology. Whether regenerating axons respond to guidance signals in the same manner as developing axons and whether these cues are still present in the adult **spinal cord** are particularly important questions for **spinal cord** regeneration. Ultimately, scientists hope to find ways to manipulate these signaling mechanisms to enhance regeneration.

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Synapse Formation

For regeneration to be successful, axons must **not** only grow but also find and connect to appropriate targets. Axons must construct the highly specialized structures that release neurotransmitters from nerve terminals. Cells that receive signals across synapses also must participate in forming new synapses at a time when they would **not** normally do so. They must assemble in precisely the right places the specialized structures necessary to respond to neurotransmitters. Finally, the developing **spinal cord** must insure that synapses of the correct type form only on proper cells and on the appropriate parts of these cells so that the neural circuits will work.

Although scientists know very little about how new synapses form in the adult mammalian **spinal cord**, they are learning how synapses develop in the skeletal neuromuscular junctions (NMJs), the synapses by which motor neurons activate muscle cells. NMJs are much more accessible for study than synapses in the **spinal cord**, and scientists have therefore used them to learn about the basic principles of synapse development and function. These nerve-muscle synapses also regenerate, which allows comparison of development and regeneration.

Although axons and muscle cells can each synthesize the specialized components they need to form synapses, development of synapses requires back-and-forth signaling between the two cell types. At the turn of the century, scientists demonstrated that regenerating motor nerves form synapses at the exact sites of former synapses, even though synapses cover only a tiny percentage of the available muscle surface. This means muscle cells must have "stop signals" that axons can recognize. Muscles also regulate their receptivity to synapse formation according to whether they already have a nerve connection. An implanted nerve will **not** form a synapse with a muscle unless the original nerve to that muscle has been removed. Muscles that have lost their nerve connections may also release molecules that entice axons to make new synapses.

In the modern era, scientists have added the tools of genetics to traditional methods of developmental neuroscience research. They can now test the role of particular molecules in synapse formation by creating mutant mice, such as "knockout mice," that lack a particular protein. Studies with knockout mice have shown how a protein called agrin helps the developing muscle aggregate molecules called acetylcholine receptors at the synapse where they are needed. Acetylcholine receptors enable muscle cells to respond to the neurotransmitter acetylcholine, which is released from the nerve terminal. Agrin knockout mice died before birth or were stillborn because of defective NMJs. Interestingly, inactivating the agrin gene **not** only affected muscle, but also the perturbed axons. This reflects the complex interactive nature of the signaling process between axons and their targets, which scientists are just beginning to understand. Scientists are now creating genetically altered mice to study other molecules that control the development of the NMJ.

In many ways, synapses within the brain and **spinal cord** resemble the NMJ, but **not** completely. Some, but **not** all, of the molecules that control development of the NMJ are present in the developing CNS. Each muscle cell receives synapses from only one axon, and all of these use the same neurotransmitter (acetylcholine). A single **spinal cord** nerve cell, on the other hand, may receive thousands of synapses from nerve cells of the brain and **spinal cord** and from sensory nerves of the body. Each **spinal cord** neuron may also respond to several different neurotransmitters. So, while **spinal cord** synapses and NMJ probably share the same general principles, the **spinal cord** must need additional signals to form synapses.

Understanding synapse formation is becoming increasingly important as the prospect improves for obtaining survival and growth of **spinal cord** cells after **injury**. So far, nerve fibers regrowing in **experimental animal** models of **spinal cord** regeneration have developed few new synapses, and this may be the limiting factor in recovery of movement. Understanding how synapses develop may reveal whether **spinal** regeneration stops because regenerating axons lack the ability to form synapses or whether nerve cells below the lesion are unreceptive to synapse formation. This may lead to ways of encouraging the formation of new synapses by regenerating fibers.

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Basic Regeneration Studies

Scientists have long known that nerve cells outside the brain and **spinal cord** can regenerate, but they believed that nerve cells in the CNS of adults could **not** regrow. In the early 1980s, experiments in the **spinal** cords of animals showed that CNS neurons can regrow under certain conditions. These experiments were inspired by the idea that adult CNS cells might be able to grow if given a permissive growth environment. Scientists grafted segments of sciatic nerve -- a peripheral nerve that can regenerate -- to the **spinal cord**, circumventing the lesion site. Some nerve cells, usually from near the site of the lesion, grew axons through the nerve bridges as far as 3 or 4 centimeters and reached the other end of the bridge. Some nerve cells in the lower parts of the brain also grew into the graft. Because of the complexity of the **spinal cord**, researchers could **not** accurately assess whether regrowing nerve cells made functional synapses or exactly what about the sciatic nerve bridges was "permissive." For this reason, some scientists turned to another model system to study CNS regeneration--the retina.

The retina of the eye is an outpost of the brain. Like the **spinal cord**, it is part of the CNS. Retinal neurons called ganglion cells carry signals from the retina to the brain. Their axons, together with supporting cells, form the optic nerve. Cutting or crushing the optic nerve, and thus the axons of the retinal ganglion cells, has become an important model for **injury** and regeneration in the CNS.

Retinal ganglion cells normally do **not** regenerate after the optic nerve has been transected. In early experiments, scientists placed peripheral nerve bridges from the site of damage in optic nerves (usually near the retina) to appropriate targets in the brain. The nerve grafts bypassed the problem of pathfinding by funneling growing axons directly to the correct region of the brain. Some ganglion cell axons grew distances equivalent to nearly twice their normal length. However, at best only a small percentage of axons regrew in these experiments since most cells died soon after transection. Some axons did penetrate the brain and make synapses, restoring the simple reflex response of pupils to light and the animals' light-avoidance behavior. Axons that reached the brain found the appropriate layers and parts of cells in the brain, but failed to recreate the proper, orderly representation of the visual world on the brain.

These retinal regeneration experiments raised many questions. What makes peripheral nervous system tissue supportive for growth? Are there growth factors in the nerve grafts that are **not** available in the adult CNS, or are growth inhibitors normally present in the adult CNS absent from the grafts? Why do so many ganglion cells die and so few regenerate? Do intrinsic genetic programs of these cells affect success and failure? How does regeneration resemble and differ from development? Experiments are beginning to answer these kinds of questions.

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Trophic Factors

For nerve cells to regenerate axons, they must first survive the **injury**. Trophic factors are signals that promote the survival and growth of nerve cells. The classical studies of trophic factors in development showed that nerve cells become dependent on these substances during the period when they specialize and begin to connect with their targets. The developing nervous system produces many more nerve cells than the adult nervous system needs. Cells compete with one another to obtain trophic factors supplied by appropriate target cells. Those neurons that do **not** succeed in competing for appropriate connections die through apoptosis.

The first trophic factor isolated was NGF (nerve growth factor). NGF is essential for the survival of some types of nerve cells in the PNS. Withdrawing NGF from peripheral neurons in culture is an important way of studying apoptosis in nerve cells. In the last several years, scientists have found that trophic factors are important in the development of the CNS as well. At the workshop, participants reported experiments suggesting that there are important differences in the trophic factor requirements of central and peripheral nerve cells. Those differences may help explain why CNS nerve cells do **not** regenerate.

Here again, retinal ganglion cells are favorable subjects for CNS research. Scientists developed methods to isolate these cells with 99 percent purity, allowing precise studies of the factors these cells need to survive and grow. The scientists then attempted to sustain these cells in culture using trophic factors that the cells might encounter in their normal course from the retina to the brain. None of these factors alone was sufficient for more than 1 percent of retinal ganglion cells to survive for even 3 days.

Studies in peripheral nerve cells have shown that activating the cyclic AMP "second messenger" system augments the effects of trophic factors. Cyclic AMP is a small molecule that carries messages from cell surface receptors activated by "first messengers" (hormones, neurotransmitters, or other signals) to sites within the cell. Like other

second messenger systems, this biochemical pathway allows a single first messenger to control several cellular processes and helps in regulating and integrating the many signals cells receive. Although activating the cyclic AMP second messenger pathway with the drug forskolin did **not** sustain retinal ganglion cells in culture, and trophic factors alone were insufficient, the two combined saved more than a third of the cultured cells. Combining multiple trophic factors with forskolin allowed survival of more than half of the cultured cells for more than a month. Adding other, as yet unpurified, factors boosted survival to more than 80 percent.

These experiments suggest that combinations of trophic factors may be essential for survival of CNS neurons. Another important insight is that the responsiveness of CNS cells to trophic factors is **not** static, but can change depending on the level of second messengers. Electrical activity and signals from other cells stimulate second messenger systems, and these influences change dramatically for cells below a **spinal cord injury**. Administering trophic factors and controlling responsiveness to these factors may promote nerve cell survival in the damaged **spinal cord**. However, these powerful and poorly understood substances can also have serious side effects.

Scientists are **not** yet certain whether adult **spinal cord** nerve cells need combinations of trophic factors, which trophic factors affect which cell types, or what controls the cells' sensitivity to these factors. In one important finding from the retina culture experiments, scientists learned that survival and axon growth were always coupled; that is, any interventions that allowed cells to survive also prompted them to extend their axons.

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Intrinsic Growth Programs

Nerve cells' intrinsic capacity to grow is another factor that may contribute to the success or failure of regeneration. Scientists have studied intrinsic growth capacity by comparing cells from young animals to those from older ones. Very young animals generally recover more completely from CNS damage than do adult animals. By placing the retina and the tectum (the brain area to which retinal ganglion cells connect) from animals together in culture, scientists demonstrated that regeneration in culture is also age-dependent. They then independently varied the age of the tectal and retinal pieces placed together in culture to determine to what extent each contributed to the failure to grow in older animals. The results showed two major reductions in the ability of ganglion cells to grow as the **animal** ages. The first, larger reduction was due to changes in the growth capacity of the retinal cells themselves. The later, smaller reduction, was more gradual and appeared to be due mostly to changes in the target tissue. Providing growth factors partially increased survival and growth but could **not** overcome the early large decline in growth ability.

Biologists believe changes in growth capacity probably reflect changes in the specific genes that are active in each cell. Several genes were inactivated at about the time that regeneration declined, but one gene was turned off just as the capacity to regenerate was lost. Surprisingly, that gene was bcl-2, which is well-known because its product is an important regulator of apoptosis. Retinal ganglion cells taken from mice with an inactivated bcl-2 gene (bcl-2 knockout mice) did **not** show the normal sharp decline in growth ability. Even cells from the adult retina of these knockout mice could grow if they were given embryonic tissue as a target. Experiments with drugs directed at enzymes in the apoptosis pathway showed that the bcl-2 gene's effects on growth were separate from its effects on apoptosis. This gene apparently acts as a "switch" that controls axon growth in the CNS. Finding ways to control this switch may yield a new approach to therapy for **spinal cord injury** that may complement other therapies such as trophic factors. While this treatment approach appears beyond genetic technology at the moment, understanding the role played by these intrinsic programs in regulating the neuron growth will provide important insights into regeneration.

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Barriers to Growth

Scientists have now identified a long list of molecules in the adult CNS that actively inhibit growth. For example, oligodendrocytes produce a myelin-associated growth inhibitor that may be one of the most important inhibitors of growth in the adult **spinal cord**. One way these inhibitors act is by making growth cones collapse. Growth inhibition may be quite specific for each nerve cell type; that is, different cells may be most sensitive to different inhibitors. Another way inhibitors act is by modifying the extracellular matrix, the noncellular material surrounding cells through which axons must grow. For example, some substances act as "anti-adhesives," preventing growing axons from sticking to surrounding tissue, which is necessary for them to grow forward. How inhibitors block axon growth and which of the many inhibitors are clinically important following **spinal cord injury** are essential questions that

scientists are now trying to answer.

Scientists need to determine the normal physiological roles of the many substances that inhibit growth in the adult **spinal cord**. Similarities in how these inhibitors work might allow generic strategies for overcoming their effects. One possibility would be to find common pathways, such as second messenger systems, through which these factors operate. Experiments with a component of pertussis toxin (a toxin from the bacteria that causes whooping cough) suggest that this might be possible. This toxin, which affects second messengers, blocked growth cone collapse from three very different inhibitory factors (collapsin-1, thrombin, and the myelin-associated factor). Because the extracellular matrix that surrounds cells is a repository for many inhibitory substances, understanding the interaction of cells with the extracellular matrix is an important focus of research. Finally, signals that inhibit and stimulate growth might converge on common intracellular machinery so that sufficient stimulation might overcome some of the inhibition. Experiments with trophic factors in retinal ganglion cells support this idea.

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Applied Regeneration Studies

Researchers are beginning to apply knowledge about nerve growth and inhibitory factors and other aspects of neuron regeneration by testing new therapeutic approaches in **animal** models of **spinal cord injury**. The partial success of several of these **animal** experiments has led to optimism by many experts that, with the right combination of strategies, regeneration will eventually become possible in humans. However, it now appears unlikely that there will be a single magic bullet for repairing the **spinal cord**. Instead, a combination of approaches will probably be necessary.

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Transplantation

One approach for repairing **spinal** cords that is being tested in animals is to transplant cells and tissues into the damaged **spinal cord**. In particular, scientists are transplanting cells or pieces of peripheral nerves that produce substances that create an environment for axons to grow. This idea was first advocated about 100 years ago by the neurologist Ramón y Cajal. He suggested implanting cells from the PNS into the area of a CNS **injury**. Since the environment of the PNS supports axon regeneration, he believed re-creating this environment in the **spinal cord** might allow CNS axons to regrow after an **injury**. Ideally, this environment would also point growing nerves to the correct targets. Experiments with PNS transplants in rat models of **spinal cord injury** have led to axon elongation and cell body changes associated with regrowth. Transplants from the PNS also seem to reduce scarring around the **injury** that may impede regrowth. One technique tested in rats is transplanting Schwann cells -- glial cells that help myelinate axons in the PNS -- into the **spinal cord** after **injury**. These transplants supported regrowth of the damaged nerves in rats with **spinal cord injury**. Researchers are now studying **human** Schwann cells to determine if this technique will work in humans.

Another way of encouraging regeneration is to implant fetal tissue. Tissue from a growing fetus contains stem cells, progenitor cells, and many substances that support growth. Such tissue also presents fewer obstacles to growing axons. Stem cells can differentiate into several cell types, depending on the signals they receive. Transplanting them into the **spinal cord** may, with the right chemical signals, help them develop into neurons and supporting cells in the **spinal cord**, re-establishing lost circuits.

Studies in rats show that fetal transplants promote survival and regrowth of some damaged nerve cells. Transplanting fetal CNS tissue into the **spinal cord** of both mature and newborn rats yielded axon growth that terminated within a few millimeters of the border of the transplant. Researchers still need to learn exactly how fetal tissue transplants promote nerve regrowth. The transplants appear to "rescue" axons and provide a bridge across which regenerating axons can grow. While both adult and newborn rats regrew descending nerve fibers from the brain, the growth of descending pathways into the transplants was substantially greater in the newborns. This suggests that other changes in the maturing CNS, such as the production of inhibitory factors or a loss of certain axon guidance molecules, may influence axonal regrowth after **injury**.

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Trophic Factors

Using insights from retina and culture experiments, researchers are beginning to test whether trophic factors can enhance regrowth in the **spinal** cords of rats. Growth factors may be responsible for much of the nerve regeneration normally seen in the PNS and in CNS axons near transplanted PNS tissue.

Different pathways in the **spinal cord** may require particular combinations of growth factors for survival after **injury**. While nerve cells usually do **not** survive after axons have been severed close to the cell body, recent experiments in the rat **spinal cord** have shown that two trophic factors, brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3), can rescue nerve cells from which the axons have been recently severed. Although NT3 has short-term effects, BDNF can help nerve cells survive for 4 weeks or more after **injury**. When the trophic factors BDNF, NT3, and NT4 (neurotrophin 4) were combined with fetal tissue transplants, axons no longer stopped growing at the border of the transplant but instead greatly expanded the territory into which they projected.

The combination of transplants and trophic factors also led to an increase in c-jun, an important immediate early gene. Immediate early genes respond rapidly to many stimuli and regulate many cell functions. Interestingly, these experiments showed that axons from cells that use the neurotransmitter serotonin responded to trophic factors more vigorously than axons from cells that use other neurotransmitters. This illustrates the importance of finding the right combination of growth factors for each type of cell.

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Anti-Inhibitory Factors

Myelin-associated neurite growth inhibitor, which is produced by oligodendrocytes, is the most important CNS growth inhibitor so far identified. When researchers blocked this growth inhibitor with an antibody called IN-1, which binds to and masks the factor from growth cones, severed axons began extending past the oligodendrocytes and reconnecting with their targets. After this treatment, rats with severed **spinal** cords moved more normally and partially regained their contact-placing reflexes (in which rats move their legs to support their bodies when they are placed against a surface).

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Combination Therapies

Evidence that combining some therapies may have an additive effect has prompted researchers to focus effort on finding a combination that will achieve regeneration. Some combination therapies recently tested in rats have shown exciting results. One approach used neurotrophin 3, fetal cell transplants, and IN-1, the antibody to myelin-associated neurite growth inhibitor. Rats treated with this approach showed faster and more extensive recovery after **spinal cord injury** than those given any single treatment alone. Their recovered reflexes disappeared after researchers destroyed the cerebral cortex, showing that the brain, rather than reorganization within the **spinal cord**, controlled the reflexes. Researchers still need to learn if this therapy can be a general approach or if specific nerve pathways have specific requirements for growth. They also need to carefully define the time windows for effective combination treatment.

Another approach using nerve fiber transplantation combined with growth factors showed the first functional regeneration of completely transected rat **spinal** cords. Researchers carefully transplanted 18 pieces of peripheral nerves (one to three pieces for each of the normal nerve tracts) taken from the rats' chests to "bridge" 5-millimeter gaps at the T8 segment of rats' **spinal** cords. To evade inhibitory proteins from oligodendrocytes, the bridges routed regenerating axons away from white matter, where they would normally grow, and into gray matter. The researchers fixed the grafts in place with a glue based on a blood-clotting factor called fibrin. The glue also contained acidic fibroblastic growth factor, or aFGF, which enhances nerve fiber development. Finally, the scientists wired the vertebrae to keep the spine in place while the area healed.

After 3 weeks, rats that had received this type of graft began to recover function in their hind legs. Some of the treated rats regained some movement on both sides of their bodies, while others regained movement on only one side. The rats that recovered on both sides of their bodies eventually began partially supporting their weight with their hind limbs. They also displayed walking movements and contact-placing reflexes. The rats continued to improve gradually over the course of a year, though they never walked normally. Rats with bridges from white matter

to other white matter, rats in which the fibrin glue had no aFGF, and rats that did **not** receive transplants did **not** recover any function over time.

Anatomical studies of **spinal** cords from rats that recovered function after this therapy showed that the nerve fibers grew into the gray matter on the opposite side of the gap. The fibers then grew at the interface between the gray matter and the white matter, an area that corresponds to the normal corticospinal tract in rats. The degree of recovery corresponded significantly to the degree of motor fiber regeneration.

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Conclusion

Basic research has led to a better understanding of trophic factors, growth barriers in the CNS, and the intrinsic capacity of nerve cells to grow. These insights are being applied in **animal** models of **spinal cord injury** using transplantation, trophic factors, and anti-inhibitory molecules. The exciting results of strategies that combine these interventions suggest that such approaches will ultimately prove the most successful for regenerating **spinal cord** pathways in humans. Developmental studies of cell specialization, axon growth and pathfinding, and synapse formation are leading to promising new avenues for improving on these combination approaches.

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Current Interventions

While the possibilities for new therapies deserve much attention, research also may be able to improve existing strategies, including drug therapy, neural prostheses, and rehabilitation.

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Drug Therapy

Effective drug therapy for **spinal cord injury** first became a reality in 1990, when methylprednisolone, the first drug shown to improve recovery from **spinal cord injury**, moved from clinical trials to standard use. The NASCIS II (National Acute **Spinal Cord Injury** Study II) trial, a multicenter clinical trial comparing methylprednisolone to placebo and to the drug naloxone, showed that methylprednisolone given within 8 hours after **injury** significantly improves recovery in humans. Completely paralyzed patients given methylprednisolone recovered an average of about 20 percent of their lost motor function, compared to 8 percent recovery of function in untreated patients. Paretic (partially paralyzed) patients recovered an average of 75 percent of their function, compared to 59 percent in people who did **not** receive the drug. Patients treated with naloxone, or treated with methylprednisolone more than 8 hours after **injury**, did **not** improve significantly more than patients given a placebo.

The successful clinical trial of methylprednisolone revolutionized thinking in the medical community. The trial showed conclusively that there is a window of opportunity for acute treatment of **spinal cord injury**. Some doctors are now using this idea to guide surgical treatment as well as drug therapy. Today, most patients with **spinal cord** injuries receive methylprednisolone within 3 hours after **injury**, especially if the **injury** is severe. This shows that emergency rooms and acute care facilities are aware of the drug's value and are capable of providing rapid treatment for **spinal cord** trauma. Success in delivering this drug on a widespread basis shows that health care systems are capable of providing rapid treatment. The NASCIS II trial also proves that well-designed trials of acute therapies for **spinal cord injury** are feasible and provides a model for testing other interventions.

Other drugs are now being tested in clinical trials. A recently completed trial suggested that 48-hour regimen of methylprednisolone may be warranted in some patients. Preliminary clinical trials of another agent, GM-1 ganglioside, have shown that it is useful in preventing secondary damage in acute **spinal cord injury**, and other studies suggest that it may also improve neurological recovery from **spinal cord injury** during rehabilitation.

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Neural Prostheses

While it may eventually become possible to help injured **spinal** cords regrow their connections, another approach is to compensate for lost function by using neural prostheses to circumvent the damage. These sophisticated electrical

and mechanical devices connect with the nervous system to supplement or replace lost motor and sensory functions. Neural prostheses for deafness, known as cochlear implants, are now in widespread use in humans and have had a dramatic impact on the lives of some people. The first prostheses for **spinal cord** injured patients are now being tested in humans. One device, a neural prosthesis that allows rudimentary hand control, was recently approved by the United States Food and Drug Administration (FDA). This prosthesis has been experimentally implanted in more than 60 people. Patients control the device using shoulder muscles. With training, most patients with this device can open and close their hand in two different grasping movements and lock the grasp in place by moving their shoulder in different ways. These simple movements allow the patients to perform many activities of daily life that they would otherwise be unable to perform, such as using silverware, pouring a drink, answering a telephone, and writing a note.

Neural prostheses are complex and contain many intricate components, such as implantable stimulators, electrodes, leads and connectors, sensors, and programming systems. There are many technical considerations in selecting each component. The electronic components must be as small as possible. Biocompatibility between electrodes and body tissue is also necessary to prevent the person from being harmed by contact with the device and to prevent the device from being harmed by contact with the person. Other challenges include finding ways to safely send currents into the body, to reliably record neural activity, and to cope with changes in muscle properties due to the **injury**. Neural prostheses also must be evaluated for usefulness and long-term safety.

Although many years of intensive study have contributed to the development of the prostheses now being tested, they are really the first generation of useful devices. Better materials and enhanced technology can refine these devices to provide much better function. Among the recent technical advances are extremely small probes that fit 16 electrodes on a shaft finer than a **human** hair. Integrated into a neural prosthesis, this type of electrode could provide extremely selective stimulation within the CNS, allowing the patient much more refined muscle control and a greater range of function. Future clinical development may allow easier, faster, and more natural movements; improve the longevity and reliability of components; and eliminate external cabling systems and external mounting of sensors.

Further research to improve components and increase understanding of brain circuits may yield prostheses that can provide sensory information to the brain. This will improve both the safety of the devices and the patient's performance of tasks. Devices now being developed may eventually enable people with **spinal cord injury** to stand unassisted and to use signals from the brain, instead of muscles, to control movement. Other types of neural prostheses currently being developed around the world aim to improve respiratory functions, bladder control, and fecal continence. Ultimately, researchers may be able to harness reflexes or the innate pattern-generating abilities of the **spinal cord's** central pattern generators to help people with **spinal cord** injuries walk.

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Rehabilitation

Rehabilitation techniques can greatly improve patients' health and quality of life by helping them learn to use their remaining abilities. Studies of problems that **spinal cord injury** patients experience, such as spasticity, muscle weakness, and impaired motor coordination, are leading to new strategies that may overcome these challenges. As they gain a better understanding of what causes these problems, physicians are learning how to treat them, sometimes using drugs already available for other health problems.

Spasticity, in which abnormal stretch reflexes intensify muscle resistance to passive movements, often develops after **spinal cord injury**. Several factors may contribute to spasticity. Changes in the strength of connections between neurons or in the neurons themselves may alter the threshold of the stretch reflex. **Spinal cord injury** also may release one type of interneurons from control by a class of neurotransmitters that includes serotonin and norepinephrine. This change in the balance of neurotransmitters may increase these neurons' excitability and enhance stretch reflexes. Drugs that mimic serotonin can partially restore reflexes, a finding that supports this neurotransmitter theory. Another possible cause of spasticity is that the reactions of pressure receptors in the skin may become stronger, causing muscle spasms that may grow stronger with time. Interneurons activated by NMDA receptors also may contribute to spasticity. NMDA receptors probably help adjust the strength of connections in the brain during learning. Researchers have found that a class of drugs that blocks NMDA receptors can restore stretch reflexes to almost normal strength.

The muscle weakness that frequently occurs after **spinal cord injury** may result from a loss of excitatory signals from the descending tracts. Abnormal patterns of motor activation in muscles may also contribute by making muscles less efficient so that they tire more easily. Loss of serotonin and related neurotransmitters may disrupt the process that controls how much each nerve cell's activity increases with increasingly strong stimuli. Restoring

normal neurotransmitter signals with drugs may partially relieve these problems. Some muscle weakness may also result from abnormal patterns of muscle usage or from changes in muscle properties, including muscular atrophy and growth of connective tissue.

Scientists believe another common motor problem, muscle incoordination, may result in part from the substantial brain reorganization that occurs after **injury** to the CNS. With a better understanding of how the **spinal cord** changes following **injury**, researchers may be able to use drugs or physical therapy to promote reorganization when it is useful and block it when it is harmful.

Rehabilitation strategies will continue to play an important role in the management of **spinal cord injury**, and they will increase in importance as the ultimate goal of functional **spinal cord** regeneration is realized. Studies in animals with **spinal cord** injuries have shown that recovery of movement is linked to the type of training the animals receive. Physical therapy and other rehabilitation strategies also are crucial for maintaining flexibility and muscle strength and for reorganizing the nervous system. These factors will be vital to recovering movement following regeneration as well as maximizing the use of undamaged nerve fibers.

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Conclusion

Therapies for **spinal cord injury** have improved substantially in the last few years. Drugs for treatment of acute **injury**, neural prostheses, and advanced rehabilitation strategies are improving the survival and quality of life for many patients. However, there are still many opportunities for improvement. These include finding ways to build on CNS reorganization and comparing the usefulness of different rehabilitation strategies. Investigators must also develop improved **animal** models for **spinal cord injury** to allow testing of new or improved therapies.

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PRECLINICAL AND CLINICAL TESTING OF NEW THERAPIES

Researchers have identified a wide variety of potential therapies for **spinal cord injury**. To efficiently evaluate these therapies, however, investigators need to carry out well-designed preclinical and clinical trials that will reveal the benefits and drawbacks of each strategy.

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Preclinical Testing

Each of the factors contributing to secondary damage presents opportunities for therapeutic intervention. Among these are neuroprotective drugs that might be combined with or even replace methylprednisolone. These drugs include antioxidants, calcium blockers, and drugs that control excitotoxicity. Drugs that enhance axon signaling, such as 4-aminopyridine, form another category of potential therapies. Drugs designed to promote regeneration by capitalizing on newfound knowledge about guidance molecules, trophic factors, and growth-inhibiting substances make up a third class. Other kinds of interventions, such as transplantation, peripheral nerve grafts, hypothermia (cooling), and combinations of therapies also show promise in regrowing **spinal cord** tracts and promoting recovery of function.

While all of these potential therapies appear promising, **not** all are at the same stage of development. Some neuroprotective drugs, including certain antioxidants and antiexcitotoxic drugs, are already being tested in humans for other purposes. Recently discovered molecules, such as those that control axon guidance, will require a great deal of basic and applied research before they can be developed into useful drugs. With so many potential therapies for **spinal cord injury**, investigators must carry out efficient preclinical tests to ensure that the most effective therapies proceed as rapidly as possible to clinical trials and, ultimately, to proven safety and usefulness. New **animal** models and better ways to monitor the success of treatments are essential to this process.

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Clinical Trials

Randomized, controlled **human** clinical trials are the "gold standard" for revealing the benefits and drawbacks of a

therapy. However, such trials are usually very expensive, and they are unlikely to yield useful results without adequate preclinical study. Clinical trials that do **not** yield clear answers are an enormous waste of resources. Physicians conducting clinical trials also must ensure that they do no harm to the patients in their study. The Belmont report of 1978, which guides **human** medical research ethics in the United States, reaffirmed that the rights of individuals participating in clinical trials must take precedence over the potential benefit to society as a whole. This restricts randomized trials to those therapies that have shown potential usefulness in systematic preclinical studies. Only with good preclinical data can researchers predict which treatment regimens might be useful and whether new therapies can be combined with standard therapy.

A clinical trial involves hundreds of components, all of which are important to its success. Seven components essential to a good trial include the rationale, or reasons the trial should be carried out; the design, which should compare different therapies (or therapy and placebo); the inclusion and exclusion criteria determining which patients should enter the trial; the use of randomization or bias control measures; the number of patients to be tested in order to produce clear results; carefully defined outcome events (that is, measures of how well patients recover); and the analysis of the data. For a clinical trial to be justified, physicians should ideally be in a state of "equipoise" in which they are **not** sure whether a treatment works or **not**. If they are certain a treatment works, it is unethical to withhold it from patients. Yet, without a reasonable expectation that patients will benefit, it is difficult to justify the risks.

There are three phases of systematic clinical testing in the United States. Phase I trials determine the criteria for safe and effective use of the therapy. These trials usually involve small numbers of patients and test the therapy in a range of doses. It is important to make this phase as extensive as necessary to eliminate unknown factors that can confound the results of later, more expensive phase II and III trials. Phase II trials establish whether the therapy, at safe and optimal doses, works for the disease. These trials should also help define factors such as which patients might benefit from the therapy. Finally, Phase III trials compare the new therapies to other therapies and/or to placebo. These trials are usually very large, as they must involve enough patients to reasonably show the drug's benefits and potential adverse reactions. A company must obtain phase III data before applying for FDA approval of a new drug.

The NASCIS trial that established the benefits of methylprednisolone is a model of an efficient phase III clinical trial for **spinal cord** therapy. This efficiency resulted from the trial's design, which used one placebo control group compared to two therapies: methylprednisolone and naloxone. This design made optimal use of resources, with a minimal number of patients given placebo. The NASCIS II trial also revealed that most patients improve somewhat, regardless of whether or **not** they receive methylprednisolone -- knowledge that is important for designing future clinical studies. Because methylprednisolone reduces disability, clinical trials can no longer use placebo controls because it would be unethical to withhold the drug from patients. Instead, new therapies must be compared to methylprednisolone, the best standard therapy.

A special problem in testing therapies for **spinal cord injury** is that most studies thus far have found combination therapies to be the most effective strategies. The need to test several therapies together complicates and can confound traditional clinical trial strategies. Investigators must find effective ways to deal with this problem to test many of the promising therapies for **spinal cord injury**.

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Conclusion

Researchers have identified a wide variety of potential therapies for **spinal cord injury**. To efficiently evaluate these therapies, however, investigators need to carry out well-designed, preclinical and clinical studies. Key elements include cooperation between multiple independent research centers, strategic trial design, and well-defined criteria for selecting potential therapies to be tested. The success of the methylprednisolone trial and advances from the basic science realm have stimulated the pace of research on treating **spinal cord injury**. With properly designed trials, potential therapies can be efficiently tested so they can help people with **spinal cord** injuries as soon as possible.

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Conclusion

Spinal cord injury research has now come of age. Because of general progress in neuroscience, as well as specific advances in **spinal cord injury** research, researchers can test new ideas about how changes in molecules, cells, and their complex interactions in the living body determine the outcome of **spinal cord injury**. Scientists are learning, for example, how processes such as oxidative damage, excitotoxicity, and apoptosis contribute to **spinal cord injury** and how this damage might be minimized. Inspired by demonstrations that **spinal cord** nerve cells can regrow, researchers are learning to manipulate trophic factors, intrinsic growth programs, and growth inhibitors to

encourage regeneration.

One of the most exciting aspects of the workshop was the potential for applying findings from other fields, such as development, immunology, and stroke research, to **spinal cord injury**. There is increasing recognition that similar processes contribute to a diverse range of neurological disorders, including **spinal cord injury**, stroke, brain trauma, and neurodegenerative diseases. New insights about how the nervous system develops are also suggesting ways to encourage regeneration. Researchers may debate how directly these insights will apply to the adult **spinal cord**, but they agree that testing these hypotheses in **animal** models of **spinal cord injury** ultimately will lead to better treatments.

Overcoming **spinal cord** injuries will require general progress in many fields of neuroscience as well as specific studies in **animal** models of **spinal cord injury** and in patients themselves. Key areas for research include:

- Secondary damage and intrinsic repair processes, including oxidative damage, excitotoxicity, calcium-mediated damage, proteases, apoptosis, immune responses, stem cells, and plasticity and reorganization.
- Development and regeneration, including trophic factors, axonal pathfinding, growth inhibitors, and synapse formation.
- Applied studies in **animal** models of **spinal cord injury**, including tests of trophic factors and grafting and transplantation strategies.
- Clinical research in **human** patients, including studies to describe anatomical and functional changes that follow **spinal cord injury**, to refine existing supportive and rehabilitation therapies such as neural prostheses, and to test new therapies that emerge from basic and applied research.

Researchers are wary of giving people false hopes that a magic bullet for curing **spinal cord injury** is just around the corner. However, with accelerating progress in scientific research, there is renewed vitality and growing optimism that, with continued effort, the problems of **spinal cord injury** will be overcome.

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Glossary of Major Terms

antioxidants - natural body chemicals or drugs that reduce oxidative damage, such as that caused by free radicals.

apoptosis - programmed cell death, or "cell suicide"; a form of cell death in which a controlled sequence of events (or program) leads to the elimination of cells without releasing harmful substances into the surrounding area. Many types of cell damage can trigger apoptosis, and it also occurs normally during development of the nervous system and other parts of the body. Strictly speaking, the term apoptosis refers only to the structural changes cells go through, and programmed cell death refers to the complete underlying process, but the terms are often used synonymously.

ascending pathways - nerve pathways that go upward from the **spinal cord** toward the brain and carry sensory information from the body.

astrocytes - the largest and most numerous of the supporting, or glial, cells in the brain and **spinal cord**. Astrocytes (meaning "star cells" because of their shape) contribute to the blood-brain barrier, help regulate the chemical environment around cells, respond to **injury**, and release regulatory substances that influence nerve cells.

autonomic dysreflexia - a potentially life-threatening increase in blood pressure, sweating, and other autonomic reflexes in reaction to bowel impaction or some other stimulus.

axon - long nerve cell fibers that conduct electrical impulses. Axons contact other nerve, muscle, and gland cells at synapses and release neurotransmitters that influence those cells.

axonal transport - the process by which nerve cells move substances from the cell body down the axon, and from the end of the axon back to the cell body. Transport down the axon is necessary because axons cannot synthesize many substances, such as proteins, that they need. Transport back to the cell body recycles substances and also carries signals taken up by axon terminals, such as trophic factors, to the cell body where they can affect cellular processes.

blood-brain barrier/blood-spinal-cord barrier - barriers, largely formed by endothelial cells that line blood vessels, that restrict the entry of circulating substances and immune cells into the brain and **spinal cord**. Trauma may compromise these barriers and contribute to further damage in the brain and **spinal cord**. These barriers also

prevent entry of some potentially therapeutic drugs.

C. elegans - a tiny nematode worm that has become a favorite **experimental** tool for scientists. The adult worm has only about 300 nerve cells, each recognizable, and is amenable to genetic manipulations. Many genes discovered in this worm are closely related to genes in mammals.

cell adhesion molecules - molecules on the outside surfaces of cells that bind to other cells' adhesion molecules or to the extracellular matrix (the material surrounding cells). Cells change their cell adhesion molecules during development and in adulthood when they encounter foreign molecules, sense damaged tissue in the vicinity, or detect cytokines. Cell adhesion molecules influence many important functions, such as the entry of immune cells into the damaged **spinal cord** and the pathfinding of growing axons.

central cord syndrome - central **cord** syndrome affects the cervical region of the **cord** and results from focused damage to the corticospinal tracts. Patients with this type of **injury** often spontaneously and rapidly recover a great deal of function within days or weeks after **injury**.

central nervous system (CNS) - the brain, **spinal cord**, and retina.

clinical trials - systematic studies in **human** patients aimed at determining the safety and effectiveness of new or unproven therapies. There are three phases to systematic clinical testing in the United States. Phase I trials determine the criteria for safe and effective use of the therapy. Phase II trials, using relatively small groups of patients, establish whether the therapy, at safe and optimal doses, works. Phase III trials, which usually require much larger numbers of patients, compare the new therapies to established therapies and/or placebo.

Computed tomography (CT) - a diagnostic imaging method in which x-ray measurements from many angles are combined in an image. CT scans help physicians evaluate bone structures and bleeding within the skull and spine.

contusion - a bruising **injury**. **Spinal cord** contusions result in a cavity or hole in the center of the **spinal cord**. Myelinated axons typically survive around the perimeter of the **spinal cord**, and the dura may even remain unbroken by the **injury**.

corticospinal tracts - the nerve fibers that carry signals from motor control areas of the brain's cerebral cortex to the **spinal cord**.

cytokines - chemical messenger molecules by which immune cells communicate with one another and with other cells. Some cytokines are also used by nerve cells as messenger molecules.

cytoskeleton - the internal scaffolding of cells. The cytoskeleton determines cell shape, organizes structures within cells, and helps cells and growth cones of developing axons move.

dendrites - the tree-like branches from nerve cell bodies that receive signals from other nerve cells at synapses.

descending pathways - nerve pathways that go down the **spinal cord** and allow the brain to control movement of the body below the head.

dorsal - refers to the back of an organism, as in the dorsal fin of a shark.

excitotoxicity - excessive release of neurotransmitters causing damage to nerve and glial cells. Excitotoxicity probably contributes to damage following CNS trauma and stroke and may also contribute to some neurodegenerative diseases. Glutamate, the most prevalent neurotransmitter by which nerve cells excite (activate) one another, is usually the culprit.

extracellular matrix - the material that surrounds cells. Important regulatory molecules in the extracelluar matrix promote, inhibit, or guide growth of axons.

free radicals - highly reactive chemicals that attack molecules crucial for cell function by capturing electrons and thus modifying chemical structures.

glia - supporting cells of the nervous system. Glial cells in the brain and **spinal cord** far outnumber nerve cells. They **not** only provide physical support, but also respond to **injury**, regulate the chemical composition surrounding cells, participate in the blood-brain and blood-**spinal-cord** barriers, form the myelin insulation of nervous pathways, help guide neuronal migration during development, and exchange metabolites with neurons. They may also produce substances that help and hinder regeneration in the **spinal cord**. The major types of glial cells in the CNS are astrocytes, oligodendrocytes, and microglia.

gray matter - the parts of the brain and **spinal cord** composed mainly of cell bodies and dendrites. The gray matter of the **spinal cord** lies in a butterfly-shaped region in the center of the **cord**.

growth cones - specialized structures at the tips of growing axons. Growth cones sense guidance signals in their environment and "steer" growing axons.

immediate early genes - genes that respond quickly to many types of stimuli and control the activity of other genes. They participate in the cellular programs that control regeneration and apoptosis.

interneurons - neurons confined wholly with the **spinal cord**, as compared with sensory and motor neurons whose axons project outside the **cord**. **Spinal cord** interneurons help integrate sensory information and generate coordinated muscle commands.

ischemia - inadequate blood flow. The brain and **spinal cord** are easily damaged by ischemia.

knockouts - genetically engineered mutant animals (usually mice) in which a particular gene has been inactivated.

magnetic resonance imaging (MRI) - a type of diagnostic imaging that relies upon the interactions of magnetic fields and radio frequency radiation with body tissues. MRI is better than CT scans for viewing soft tissue.

methylprednisolone - a steroid. Methylpredisolone administered within 8 hours of acute **spinal cord** trauma is the first drug shown to improve recovery from **spinal cord injury**.

motor neurons - nerve cells whose axons contact and control skeletal muscles.

myelin - the electrically insulating coating around axons that gives white matter its whitish appearance. Myelin increases the speed and reliability of signal transmission along nerve fibers. In the CNS, oligodendrocytes, a type of glial cell, wrap myelin around axons. In the PNS, Schwann cells generate myelin.

NASCIS - National Acute **Spinal Cord Injury** Study; NASCIS II was the clinical trial that demonstrated the effectiveness of methylprednisolone for acute **spinal cord injury**.

necrosis - a type of cell death in which cells swell and break open, release their contents and can damage neighboring cells and provoke inflammation.

neural prosthesis - an electronic and/or mechanical device that connects with the nervous system and supplements or replaces functions lost by disease or **injury**.

neuron - a nerve cell.

neurotransmitter - chemicals released by nerve cells at synapses that influence the activity of other cells. Neurotransmitters may excite, inhibit, or otherwise influence the activity of cells.

oligodendrocytes - a type of glial cell in the brain and **spinal cord**. Oligodendrocytes wrap axons with myelin which improves the speed and reliability of impulse conduction. These cells also produce substances that inhibit the regeneration of axons in the adult CNS.

oxidative damage - damage to cells caused by oxidants, or chemicals that capture electrons from other substances. Some oxidants are generated in the normal course of oxidative metabolism (energy production). The production of these substances increases during certain diseases and following trauma or stroke and contributes to the secondary damage that follows these events.

placebo - an inert substance or inactive treatment given instead of a therapy that is being evaluated.

plasticity - the ability of the nervous system to change with experience.

programmed cell death - apoptosis.

proteases - enzymes that degrade proteins. Proteases are important regulators of cell function, but inappropriate activation of proteases resulting from trauma can be harmful.

receptors - molecules, usually found on the surfaces of cells, that enable cells to respond to neurotransmitters, hormones, and other messenger molecules. Receptors may act directly by opening ion channels in the cell membrane that are part of the same receptor molecule, or indirectly by activating second messenger systems that

go on to affect various processes in the cell. The term receptor also refers to cells or structures that receive sensory information, such as pain receptors and light receptors in the eye.

retina - the neural retina, at the back of the eye, which is part of the CNS. Rods and cones of the retina detect light. Other nerve cells within the retina perform the first stages of analysis of the visual world. The axons of retinal ganglion cells, with supporting cells, form the optic nerve and carry these signals to the brain.

Schwann cells - glial cells in the PNS that wrap nerve fibers with myelin and also secrete regulatory factors.

second messenger system - a kind of biochemical pathway within cells that is controlled by "first messengers," such as hormones or neurotransmitters, that bind to receptors on the cell surface. Second messengers diffuse within the cell and alter cell behavior. They amplify signals, allow a single first messenger to control several cellular processes, and help integrate the many signals cells receive.

secondary damage - damage that continues in the hours following the initial trauma.

spinal cord segments - divisions of the **spinal cord** along its length. Each **spinal** segment sends a pair of motor and sensory nerves to the body. Higher segments control movement and sensation in upper parts of the body, while lower segments control lower parts of the body.

spasticity - a state of increased muscular tone in which abnormal stretch reflexes intensify muscle resistance to passive movements.

synapse - the functional connection between a nerve cell axon and target cells, which may be other nerve cells, muscle cells, or gland cells. At the synapse the axon releases a chemical neurotransmitter that diffuses across a tiny gap and binds to receptors (molecules on the surface of the target cell) that then change the target cell's behavior. Synapses may be excitatory (increasing a target cell's electrical activity), inhibitory (reducing a target cell's activity) or have more complex influences (such as adjusting the sensitivity of cells to other signals).

trophic factor - a natural cell growth and survival molecule. Neurotrophic factors are trophic factors that affect nerve cells.

ventral - toward the front of the body.

white matter - areas of the brain and **spinal cord** that contain mainly nerve fibers rather than nerve cell bodies and dendrites (gray matter). The myelin insulating covering of axons gives the whitish appearance. White matter is located in the outer portion of the **spinal cord**, and gray matter is in the center.

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Dedication

This workshop is dedicated to Dr. Richard P. Bunge.

Dr. Bunge was a major figure in **spinal cord injury** research and in modern neurobiology. Until his untimely death, Dr. Bunge was scheduled to give one of the major lectures at the workshop. Indeed, it would have been impossible to think of a meeting such as this without him. At the time of his death, he was Professor of Neurological Surgery at the University of Miami and Director of the Miami Project to Cure Paralysis. His career exemplifies the synthesis of basic and clinical research that this meeting hoped to foster. As a basic neurobiologist, he made fundamental contributions to the scientific understanding of nerve cells and their interaction with Schwann cells. At the peak of his career, he became interested in **spinal cord injury** and in the possibility of promoting repair of the **spinal cord**. With his wife and scientific partner, Dr. Mary Bunge, he moved to Miami in 1989. There, they assembled a team of scientists to investigate the basic and clinical problems related to **spinal cord injury**. His recent death is an immeasurable loss to the scientific and **spinal cord injury** communities.

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Neurology Today. 3(1):19,22, January 2003.
Susman, Edward